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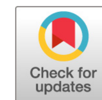

In Vitro Antibiotic Susceptibilities of *Francisella tularensis* Determined by Broth Microdilution following CLSI Methods

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ABSTRACT *In vitro* susceptibilities for 47 antibiotics were determined in 30 genetic diverse strains of *Francisella tularensis* by the broth microdilution method following Clinical and Laboratory Standards Institute (CLSI) methods. The *F. tularensis* strains demonstrated susceptibility to aminoglycosides, fluoroquinolones, and tetracyclines. There was a distinct difference in macrolide susceptibilities between A and B type strains, as has been noted previously. The establishment and comparison of antibiotic susceptibilities of a diverse but specific set of *F. tularensis* strains by standardized methods and the establishment of population ranges and $MIC_{50/90}$ values provide reference information for assessing new antibiotic agents and a baseline to monitor any future emergence of resistance, whether natural or intentional.

KEYWORDS *Francisella tularensis*, antibiotics, susceptibility testing

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Francisella tularensis is the causative agent of tularemia, a zoonotic infection in humans, usually transmitted as a result of contact with infected animals or insect bites (1). It also has potential misuse in biowarfare and bioterrorism (2). While the disease is not usually fatal, underlying medical conditions can lead to possibly fatal complications. Antibiotic therapy usually resolves the infection (1). The species is also divided into several biovars, based on metabolic differences, virulence, and geographic location (3). There is scattered information on MICs under a variety of nonstandardized testing conditions, with mostly type B (*F. tularensis* subsp. *holarctica*) strains (4–15). A more recent report utilizing standardized testing methods with a variety of North American type A strains (16) has provided some additional data; however, this study looked at only eight antibiotics representing five classes. With a lack of comparative data on type strains and with a variety of antibiotic classes, we report here specific antibiotic susceptibility results according to Clinical and Laboratory Standards Institute (CLSI) microdilution broth methodology for 30 strains of *F. tularensis* (17). This information will be highly useful as baseline data in the event of wartime or terrorist release, as well as for naturally acquired and laboratory-acquired infections.

The *F. tularensis* strains used in this study are shown in Table 1, and they were obtained from the U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID) collection and selected to represent the established biovars and geographic diversity (18). Most antibiotics were obtained from U.S. Pharmacopoeia (Rockville, MD), with the following exceptions: ceftriaxone and fusidic acid were from Sigma Chemical Co. (St. Louis, MO), cethromycin was from Advanced Life Sciences; telithromycin was from Sanofi-Aventis, garenoxacin was from Schering-Plough, gemifloxacin was from Oscient, ertapenem was from Merck, faropenem was from Replidyne, and tigecycline was from Wyeth. Most stock solutions (5 mg/ml) were prepared for each drug in the appropriate solvents, based on the current CLSI recommendations (19), and stored at −70°C until use. The amoxicillin-clavulanate (2:1) stock was 5 mg/2.5 mg per ml. The co-trimoxazole stock

TABLE 1 *F. tularensis* strain information

F. tularensis strain	Source/yr[a]	Biovar
ATCC 6223	Utah/1920	A2
ATCC 15482	Utah/1950	N (novicida)
DS89-R-54	unk/1989	A1
DS88-R-675	unk/1988	A1
DS88-R-160	unk/1988	A1
DSAL91-1623	unk/1991	B
DSAZ91-1624	unk/1991	A2
DST6755	unk	A1
LVS	Russia/unk	B
SchuS4-1	Ohio/1941	A1
Strain 425	Montana/1941	B
JAP	Japan/1957	B
38A	unk/1960	A2
HUGH	unk/1948	A1
DS87-R-200	unk/1987	A1
MAX B	unk/1953	B
DS88-R-147	unk/1988	A1
SCHERM	unk/1954	A1
Larsen NIH 38	unk/1953	A2
VT68	Vermont/1968	B
SCHU-S5	Ohio/1958	A1
MAX A	unk/1953	B
IN99-1009	Indiana/1999	B
CO01-3027	Colorado/2001	A
MA00-2987	Massachusetts/2000	A
CA02-0099	California/2002	A
KY00-1708	Kentucky/2000	B
UT02-1927	Utah/2002	A
OR01-1807	Oregon/2001	B
OK00-2732	Oklahoma/2000	A

[a]unk, unknown.

contained 5 mg/ml sulfamethoxazole and 0.26 mg/ml trimethoprim (19:1). MICs were determined by the microdilution method in 96-well plates, as previously described (20), with the exception of the addition of IsoVitaleX (Becton Dickinson) to a final concentration of 2% (17, 21). Antibiotics were serially diluted 2-fold in 50 μl of cation-adjusted Mueller-Hinton broth (CAMHB). The antibiotic range was 64 to 0.008 μg/ml, based on a final well volume of 100 μl after inoculation. The inocula were prepared by picking several colonies from 36- to 48-h chocolate agar (CA) plates grown at 35°C suspended and diluted with CAMHB to a bacterial cell density of 10^6 CFU/ml (conversion factor of 3.9×10^{10} CFU/ml/optical density at 600 nm [OD_{600}]). To each well of the 96-well plate, 50 μl of this dilution was added, for a final inoculum of approximately 5×10^4 CFU/well (5×10^5 CFU/ml). The plates were incubated at 35°C and read visually at both 24 and 48 h. It was observed that several strains grew poorly or not at all if the IsoVitaleX supplement was included in premade antibiotic susceptibility testing (AST) plates. The addition of freshly reconstituted IsoVitaleX to the CAMHB inoculum at a concentration of 4% (final concentration in wells, 2%) yielded consistent results with good growth in the control and sub-MIC antibiotic wells. The observation was that for many of the more fastidious strains, premade frozen/thawed AST plates either did not support growth, or growth was greatly slowed or reduced and generally inconsistent. It is presumed that some component(s) in the supplement required for growth for these strains is labile under freeze, storage, and thaw conditions. The susceptibility results were more consistent and reproducible for all strains when the supplement was made up fresh and included in the inoculum. Quality control of all antibiotic stocks was verified by using *Staphylococcus aureus* ATCC 29213, *Pseudomonas aeruginosa* ATCC 27853, and *Escherichia coli* ATCC 25922, according to CLSI standards (19), and read at 18 to 24 h and again at 48 h (17). All bacterial work was carried out under biosafety level 3 (BSL-3) laboratory conditions.

The broth dilution susceptibility data are presented in Table 2. Few susceptibility

TABLE 2 *F. tularensis* susceptibility values for 30 strains

Antibiotic	Range (μg/ml)	MIC_{50} (μg/ml)	MIC_{90} (μg/ml)
Amikacin	≤0.03 to 8	1	2
Gentamicin	≤0.03 to 1	0.25	0.5
Netilmicin	≤0.03 to 0.5	0.25	0.25
Streptomycin	≤0.03 to >64	4	8
Tobramycin	≤0.03 to 0.5	0.12	0.25
Azithromycin	0.12 to 16	1	1
Cethromycin	≤0.03 to 8	0.12	0.5
Telithromycin	≤0.03 to 16	0.25	1
Clarithromycin	≤0.03 to >64	0.5	4
Solithromycin	≤0.015 to 4	0.03	2
Garenoxacin	≤0.004 to 0.12	0.008	0.03
Ciprofloxacin	0.015 to 0.25	0.12	0.25
Gatifloxacin	≤0.004 to 1	0.015	0.06
Gemifloxacin	≤0.004 to 0.12	0.008	0.03
Levofloxacin	0.008 to 0.25	0.03	0.06
Moxifloxacin	≤0.004 to 0.12	0.008	0.06
Nalidixic acid	0.25 to 16	0.5	2
Ofloxacin	0.015 to 0.06	0.015	0.06
Sparfloxacin	≤0.004 to 0.06	≤0.004	0.12
Novobiocin	≤0.03 to 1	0.06	0.5
Amoxicillin-clavulanate (2:1)	0.5 to >64	16	16
Amoxicillin	4 to >64	>64	>64
Ampicillin	4 to >64	>64	>64
Penicillin G	2 to >64	>64	>64
Piperacillin	0.03 to >64	32	>64
Imipenem	0.06 to >64	0.12	8
Ertapenem	0.06 to >64	1	8
Faropenem	0.12 to 16	2	4
Meropenem	0.12 to >64	1	16
Cefepime	0.12 to >64	4	16
Ceftazidime	0.03 to >64	0.12	1
Cefotaxime	0.03 to >64	1	2
Cefotetan	0.25 to >64	2	8
Cefuroxime	0.5 to >64	4	16
Cefazolin	0.5 to >64	>64	>64
Ceftriaxone	≤0.03 to >64	0.25	2
Ceftaroline	0.06 to >8	0.25	8
Aztreonam	0.5 to >64	2	8
Sulfamethoxazole	4 to >256	128	>256
Co-trimoxazole (19:1)	0.25 to 8	1	4
Trimethoprim	0.25 to >64	4	16
Doxycycline	≤0.03 to 4	0.12	0.25
Tetracycline	≤0.03 to 1	0.12	0.25
Tigecycline	≤0.03 to 0.5	0.12	0.25
Rifampin	≤0.03 to 2	0.12	0.5
Chloramphenicol	0.25 to 8	2	4
Fusidic acid	≤0.03 to 32	2	8

breakpoints have been established for *F. tularensis*. The CLSI has developed some interpretive criteria based in part on the data presented here, other published *in vitro* distribution data, and animal efficacy studies (17). Standard testing at 35°C provided MIC_{90}s that could be interpreted as susceptible for gentamicin (Fig. 1A), streptomycin (Fig. 1B), ciprofloxacin (Fig. 1C), levofloxacin (Fig. 1D), doxycycline (Fig. 1E), tetracycline (Fig. 1F), and chloramphenicol (Fig. 1G), using the *F. tularensis* breakpoints (17). The range and MIC_{90} values for these antibiotics were in general agreement with the results from a CDC study (16). Differences observed between the two studies may be due to the greater strain distribution in this collection, particularly among the B biovar strains. Amikacin, netilmicin, tobramycin, gatifloxacin, gemifloxacin, moxifloxacin, ofloxacin, nalidixic acid, ceftazidime, cefotaxime, cefotetan, ceftriaxone, and co-trimoxazole may be active based on *Enterobacteriaceae* breakpoints (19). Some additional cephalosporins, macrolides, and rifampin may also have susceptibilities in efficacious ranges, but CLSI has no comparable breakpoints to use as a reference. Beta-lactams, carbapenems,

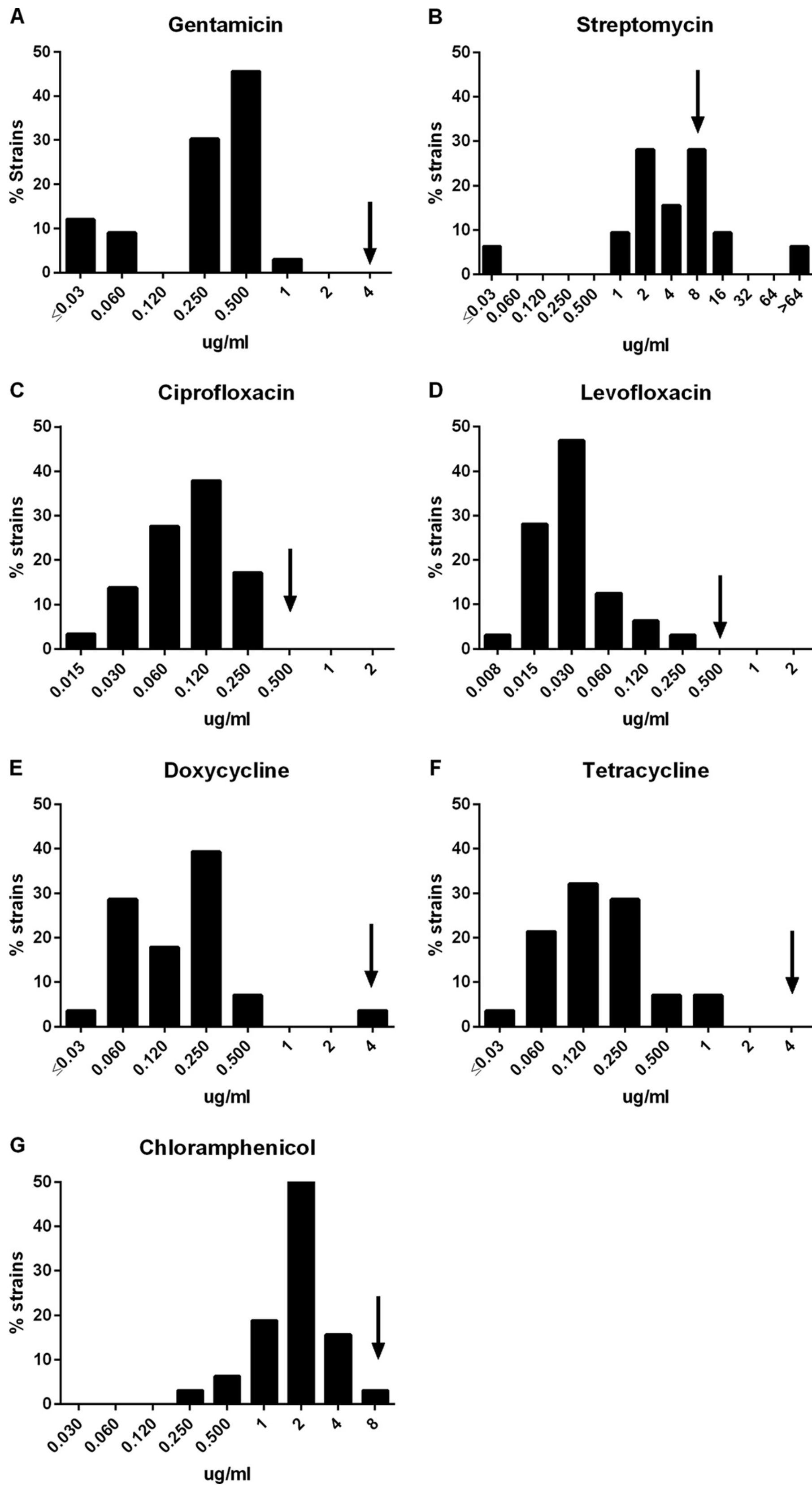

FIG 1 Thirty *F. tularensis* strain susceptibility distributions for 7 antibiotics with established CLSI breakpoints. Arrow indicates the CLSI breakpoint for each antibiotic tested: gentamicin (A), streptomycin (B), ciprofloxacin (C), levofloxacin (D), doxycycline (E), tetracycline (F), and chloramphenicol (G).

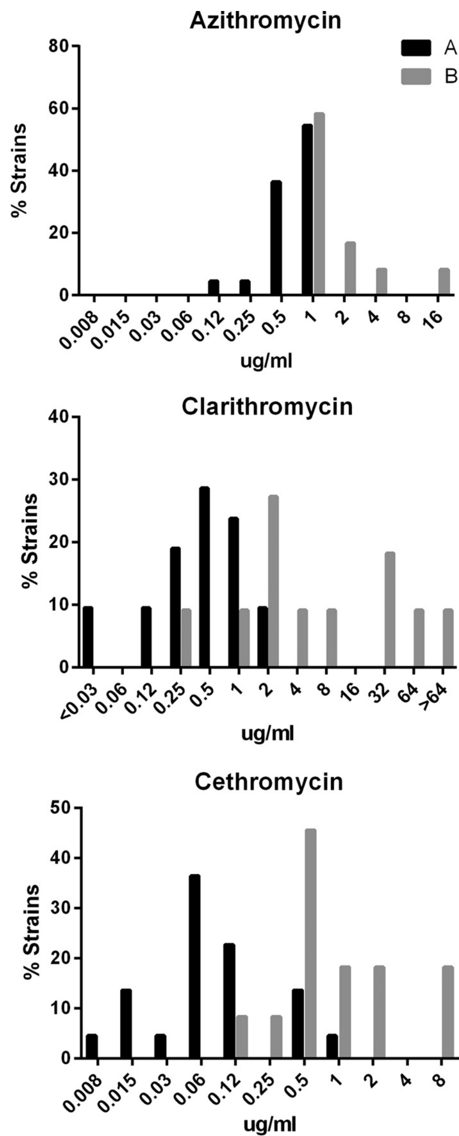

FIG 2 MIC distributions of *F. tularensis* A and B biovar strains for macrolides.

and earlier generation cephalosporin MIC_{90} values would indicate poor activity based on the same criteria. The generally high susceptibilities to beta-lactams can be attributed to a class A β-lactamase shown to be present in the genome of *F. tularensis* (22). There was a notable shift in the distribution of susceptibilities for macrolides between the A and B type strains (Fig. 2). Variation due to distinct differences between the A and B strains may cause some B strain macrolide values to be in a poor activity range. Macrolide resistance has been observed with the B strains which are prevalent in Europe and, as a result, are contraindicated for treatment (8, 10). With the exception of the macrolide distribution, no other differences were observed between A and B strains for other antibiotics.

The establishment of a broad set of antibiotic susceptibility ranges for a number of defined and archived strains of *F. tularensis* will be helpful to serve as references in future testing of new antibiotics as they are developed. The establishment of a set of MIC_{90}s will also aid in animal model-*F. tularensis* infection efficacy evaluations both in terms of which antibiotics to evaluate and the development of doses in combination with pharmacokinetic/pharmacodynamic studies.

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